

## II. AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application. Fees in the amount of \$1800.00 are included for the additional claims as follows:

# of Claims Canceled:	49	
# of New Claims:	33	
# of New Claims in Excess:	0 @ \$50.00 =	\$0.00
# of New Independent Claims:	9 @ \$200.00 =	\$1800.00
Total Fee paid:		<hr/> \$1800.00

### **Listing of Claims:**

1. (Original): A method of separating sperm cells, comprising:
  - a. obtaining semen from a male of a species of mammal which contains a plurality of sperm cells;
  - b. incubating said semen at a temperature above which sperm cell membrane lipids transition from a liquid phase to gel phase;
  - c. determining a sperm cell characteristic of a plurality of said sperm cells;
  - d. separating said sperm cells based upon said sperm cell characteristic; and
  - e. collecting separated sperm cells.
2. (Original): A method of separating sperm cells as described in claim 1, wherein said temperature above which sperm cell membrane lipids transition from a liquid phase to gel phase comprises the step of incubating said semen at a temperature above which sperm cell membrane lipids transition to a gel phase.
3. (Original): A method of separating sperm cells as described in claim 1, wherein said temperature above which sperm cell membrane lipids transition from a liquid phase to gel phase comprises the step of incubating said semen at a temperature which maintains said sperm cell membrane lipids in said liquid phase.
4. (Original): A method of separating sperm cells as described in claim 1, wherein said step of incubating said semen at a temperature above which sperm cell membrane lipids transition from a liquid phase to gel phase temperature comprises incubating said semen at a temperature between about 5° C and about 25° C.
5. (Original): A method of separating sperm cells as described in claim 1, wherein said temperature is selected from the group consisting of about 5 ° C,

about 6° C, about 7° C, about 8° C, about 9° C, about 10° C, about 11° C, about 12° C, about 13° C, about 14° C, about 15° C, about 16° C, about 17° C, about 18° C, about 19° C, about 20° C, about 21° C, about 22° C, about 23° C, about 24° C, and about 25° C.

6. (Original): A method of separating sperm cells as described in claim 1, wherein said species of mammal is selected from the group consisting of a bovine species of mammal, an equine species of mammal, an ovine species of mammal, a swine species of mammal, a canine species of mammal, a feline species of mammal, a deer species of mammal, an elk species of mammal, and a marine species of mammal.
7. (Original): A method of separating sperm cells as described in claim 1, wherein said species of mammal comprises a bovine species and wherein said step of incubating said semen at a temperature above which sperm cell membrane lipids transition from a liquid phase to gel phase temperature comprises incubating said semen at a temperature between about 17° C and about 19° C.
8. (Original): A method of separating sperm cells as described in claim 1, wherein said species of mammal comprises a bovine species and wherein said step of incubating said semen at a temperature above which sperm cell membrane lipids transition from a liquid phase to gel phase temperature comprises incubating said semen at a temperature of about 17° C.
9. (Original): A method of separating sperm cells as described in claim 1, wherein said species of mammal comprises an equine species and wherein said step of incubating said semen at a temperature above which sperm cell membrane lipids transition from a liquid phase to gel phase temperature comprises incubating said semen at a temperature of about 15° C.

10. (Original): A method of separating sperm cells as described in claim 1, wherein said step of incubating said semen at a temperature above which sperm cell membrane lipids transition from liquid phase to gel phase comprises incubating said semen at said temperature above which sperm cell membrane lipids transition from liquid phase to gel phase between about one hour to about 18 hours.
11. (Original): A method of separating sperm cells as described in claim 1, further comprising the step of transporting said semen from a first location to a second location during said step of incubating said semen at a temperature above which sperm cell membrane lipids transition from a liquid phase to gel phase.
12. (Original): A method of separating sperm cells as described in claim 1, further comprising the step of adding an antibacterial to said semen prior to said step of incubating said semen at a temperature above which sperm cell membrane lipids transition from a liquid phase to gel phase.
13. (Original): A method of separating sperm cells as described in claim 1, wherein said step of determining a sperm cell characteristic of a plurality of sperm cells within said semen comprises determining a sex characteristic of said sperm cells.
14. (Original): A method of separating sperm cells as described in claim 1, wherein said step of separating said sperm cells based upon said sperm cell characteristic comprises separating said sperm cells based upon said sex characteristic.
15. (Original): A method of separating sperm cells as described in claim 1, further comprising the step of extending semen with an extender selected from the group consisting of KMT, and INRA96.

16. (Original): A method of separating sperm cells as described in claim 1, further comprising the step of concentrating said sperm cells by removing a portion of seminal plasma.
17. (Original): A method of separating sperm cells as described in claim 1, further comprising the step of staining said sperm cells.
18. (Original): A method of separating sperm cells as described in claim 17, wherein said step of staining said sperm cells comprises staining DNA contained within said sperm cells.
19. (Original): A method of separating sperm cells as described in claim 18, wherein said step of staining DNA contained within said sperm cells comprises staining said DNA within said sperm cells with Hoechst 33342 stain.
20. (Original): A method of separating sperm cells as described in claim 19, wherein said step of staining said DNA within said sperm cells with Hoechst 33342 stain comprises incubating said sperm cells with Hoechst 33342 for a period of between about 30 minutes and about 1 hour.
21. (Original): A method of separating sperm cells as described in claim 1, wherein said step of separating said sperm cells based upon said sperm cell characteristic comprises separating said sperm cells using an instrument selected from the group consisting of a flow cytometer, and a cell sorter.
22. (Original): An artificial insemination sample, comprising:
  - a. an artificial insemination device;
  - b. a sperm cell extender; and
  - c. semen obtained from a male of a species of mammal containing a plurality of sperm cells incubated at a temperature above which sperm cell membrane lipids transition from a liquid phase to a gel phase for a period

of between one hour and 18 hours, and wherein said plurality of sperm cells are separated on the basis of a sex characteristic, and wherein a portion of said separated sperm cells are established in said sperm cell extender, and wherein said portion of said separated sperm cells established in said sperm cell extender are contained within said artificial insemination device.

23. (Original): An artificial insemination sample as described in claim 22, wherein said sperm cell extender comprises an egg yolk extender.
24. (Original): An artificial insemination sample as described in claim 22, wherein said sperm cell extender comprises an egg yolk-TRIS extender.
25. (Original): An artificial insemination sample as described in claim 22, wherein said sperm cell extender comprises substantially equal volumes of said egg yolk-TRIS extender and egg yolk-TRIS extender/12% glycerol.
26. (Original): An artificial insemination sample as described in claims 23, 24, or 25, wherein said sperm cell extender has a volume of between about 0.1 millilitre and about 2.0 millilitre.
27. (Original): An artificial insemination sample as described in claim 22, wherein said artificial insemination device comprises an artificial insemination straw.
28. (Original): An artificial insemination sample as described in claim 22, wherein said portion of said separated sperm cells established in said sperm cell extender comprise between about one hundred thousand separated sperm cells and about 25 million separated sperm cells.

29. (Original): An artificial insemination sample as described in claim 28, wherein said portion of said separated sperm cells established in said sperm cell extender contained within said artificial insemination device is frozen.
30. (Original): An artificial insemination sample as described in claim 29, wherein said portion of said separated sperm cells frozen in said sperm cell extender contained within said artificial insemination device are thawed.
31. (Original): A method of producing an artificial insemination sample comprising the steps of:
- a. obtaining semen from a male of a species of mammal which contains a plurality of sperm cells;
  - b. incubating said semen at a temperature above which sperm cell membrane lipids transition from a liquid phase to a gel phase;
  - c. determining a sex characteristic of said plurality of sperm cells;
  - d. separating said plurality of sperm cells based upon said sex characteristic; and
  - e. establishing an artificial insemination sample containing separated sperm cells capable of fertilizing at least one egg within a female of said species of said mammal.
32. (Original): A method of fertilizing a mammal comprising the steps of:
- a. obtaining semen from a male of a species of mammal which contains a plurality of sperm cells;
  - b. incubating said semen at a temperature above which sperm cell membrane lipids transition to a gel phase;
  - c. determining a sex characteristic of said plurality of sperm cells;
  - d. separating said plurality of sperm cells based upon said sex characteristic;
  - e. establishing an artificial insemination sample containing separated sperm cells capable of fertilizing at least one egg within a female of said species of said mammal;

- f. inserting a portion of said insemination sample into a female of said species of said mammal; and
  - g. fertilizing at least one egg within said female of said species of said mammal.
- 33. (Original): A method of producing an offspring mammal comprising the steps of:
  - a. obtaining semen from a male of a species of mammal which contains a plurality of sperm cells;
  - b. incubating said semen at a temperature above which sperm cell membrane lipids transition to a gel phase;
  - c. determining a sex characteristic of said plurality of sperm cells;
  - d. separating said plurality of sperm cells based upon said sex characteristic;
  - e. establishing an artificial insemination sample containing separated sperm cells capable of fertilizing at least one egg within a female of said species of said mammal;
  - f. inserting a portion of said insemination sample into a female of said species of said mammal;
  - g. fertilizing at least one egg within said female of said species of said mammal; and
  - h. producing an offspring mammal.
- 34. (Previously Presented): A process for storing unsorted spermatozoa, the process comprising
  - a. forming a sperm dispersion, the sperm dispersion comprising spermatozoa, a composition that induces sperm immotility, and an antibiotic, and
  - b. storing the sperm dispersion.
- 35. (Previously Presented): A process for storing sorted spermatozoa, the process comprising



- a. forming a sperm dispersion, the sperm dispersion comprising spermatozoa and a composition that induces sperm immotility,
  - b. sorting the sperm dispersion into separate populations, wherein the spermatozoa of one of the populations comprises at least about 65% X chromosome bearing sperm cells or at least about 65% Y chromosome bearing sperm cells, and
  - c. storing the one population at a temperature of about -4° C. to about 30° C.
36. (Previously Presented): A process for inseminating a female mammal, the process comprising inseminating a female mammal with a sperm dispersion, the sperm dispersion comprising immotile spermatozoa and a composition that induces sperm immotility.
37. (Previously Presented): A process for providing a fresh sperm dispersion for inseminating a female mammal, the process comprising:
- a. forming a sperm dispersion, the sperm dispersion comprising spermatozoa and a composition that induces sperm immotility,
  - b. placing the sperm dispersion in a container for shipment to a remote location, and
  - c. shipping the sperm dispersion in the container to a remote location within about 24 hours after forming the sperm dispersion.
38. (Previously Presented): A combination comprising:
- a. an elongated container for use in the insemination of a female mammal, and
  - b. a sperm dispersion, the sperm dispersion comprising immotile spermatozoa and a composition that induces sperm immotility, and wherein the sperm dispersion is contained in the elongated container.

39-87. (Canceled)

88. (Previously Presented): A process for inseminating a female mammal, the process comprising inseminating a female mammal with a sperm extension, the sperm extension comprising immotile spermatozoa and a composition that reduces sperm motility.
89. (New): A sperm cell suspension comprising viable spermatozoa, the spermatozoa having a motility more characteristic of epididymal spermatozoa than endogenous ejaculated spermatozoa of the same species, the concentration of spermatozoa in the suspension being less than about  $1 \times 10^6$  or at least about  $1 \times 10^8$  spermatozoa per ml.
90. (New): A sperm cell suspension comprising viable, immotile sperm, the concentration of spermatozoa in the suspension being less than about  $1 \times 10^6$  or at least about  $1 \times 10^8$  spermatozoa per ml.
91. (New): A sperm cell suspension comprising viable sperm, a motility inhibiting amount of potassium, and sodium, the concentration of spermatozoa in the suspension being less than about  $1 \times 10^6$  or at least about  $1 \times 10^8$  spermatozoa per ml and the molar ratio of potassium to sodium being greater than 1:1, respectively.
92. (New): The suspension of claim 91 wherein the molar ratio of potassium to sodium is greater than 1.25:1, respectively.
93. (New): The suspension of claim 91 wherein the molar ratio of potassium to sodium is greater than 1.5:1, respectively.
94. (New): The suspension of claim 91 wherein the molar ratio of potassium to sodium is greater than 1.75:1, respectively.
95. (New): The suspension of claim 91 wherein the molar ratio of potassium to

sodium is greater than 2:1, respectively.

96. (New): A sperm cell suspension comprising viable, immotile sperm and a DNA-selective dye.
97. (New): The suspension of claim 96, wherein the dye is a DNA selective fluorescent dye.
98. (New): The suspension of claim 96, wherein the dye is a UV excitable or a visible light excitable dye.
99. (New): The suspension of claim 96, wherein the dye is selected from the group consisting of Hoechst 33342, Hoechst 33258, SYBR-14, and bisbenzimidide-BODIPY® conjugate 6- {[3-((2Z)-2- {[1-(difluoroboryl)-3,5-dimethyl-1H-pyrrol-2-yl]methylene}-2H-pyrrol-5-yl)propanoyl]amino}-N-[3-(methyl {3-[(4-[6-(4-methylpiperazin-1-yl)-1H,3'H-2,5'-bibenzimidazol-2'-yl]phenoxy} acetyl)amino]propyl} amino)propyl]hexanamide.
100. (New): The sperm suspension of claim 90, wherein the suspension comprises a source of carbonate.
101. (New): The sperm suspension of claim 90, wherein the suspension comprises  $\text{NaHCO}_3$ ,  $\text{KHCO}_3$ , and  $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$ .
102. (New): The sperm suspension of claim 90, wherein the suspension is formed from a buffer comprising 0.097 moles/L of  $\text{NaHCO}_3$ , 0.173 moles/L of  $\text{KHCO}_3$ , 0.090 moles/L  $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$  in water.
103. (New): The sperm suspension of claim 90, wherein the concentration of spermatozoa in the suspension is at least  $1.25 \times 10^8$  spermatozoa per ml.

104. (New): The sperm suspension of claim 90, wherein the concentration of spermatozoa in the suspension is at least  $1.5 \times 10^8$  spermatozoa per ml.
105. (New): The sperm suspension of claim 90, wherein the concentration of spermatozoa in the suspension is at least  $1.75 \times 10^8$  spermatozoa per ml.
106. (New): The suspension of claim 90, wherein the concentration of spermatozoa in the suspension is less than about  $9.0 \times 10^5$  spermatozoa per ml.
107. (New): The suspension of claim 90, wherein the concentration of spermatozoa in the suspension is less than about  $7 \times 10^5$  spermatozoa per ml.
108. (New): The suspension of claim 90, wherein the concentration of spermatozoa in the suspension is less than about  $5 \times 10^5$  spermatozoa per ml.
109. (New): The suspension of claim 90, wherein the concentration of spermatozoa in the suspension is less than about  $2 \times 10^5$  spermatozoa per ml.
110. (New): The suspension of claim 90, wherein the concentration of spermatozoa in the suspension is less than about  $1 \times 10^5$  spermatozoa per ml.
111. (New): A process for staining sperm cells, the process comprising forming a staining mixture containing intact viable sperm cells, a motility inhibiting amount of potassium, and a DNA selective dye.
112. (New): The process of claim 111 wherein the staining mixture is under an atmosphere having an enriched partial pressure of  $\text{CO}_2$  relative to air.
113. (New): A process of forming a sperm cell suspension for use in cell sorting, the process comprising combining a sperm cell source with a composition which inhibits the motility of sperm cells to form a sperm cell suspension, the

concentration of sperm cells in the suspension being less than about  $1 \times 10^6$  or at least  $1 \times 10^8$  sperm cells per milliliter.

114. (New): The process of claim 113, wherein the cell suspension comprises a DNA-selective dye.
115. (New): The suspension of claim 101, further comprising a DNA-selective dye.
116. (New): A sperm cell suspension comprising viable, immotile spermatozoa, the spermatozoa having a DNA-selective dye associated with their DNA.
117. (New): The suspension of claim 116, wherein the dye is a UV excitable or a visible light excitable dye.
118. (New): The sperm suspension of claim 116, wherein the suspension comprises  $\text{NaHCO}_3$ ,  $\text{KHCO}_3$ , and  $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$ .
119. (New): The sperm suspension of claim 116, wherein the suspension is formed from a buffer comprising 0.097 moles/L of  $\text{NaHCO}_3$ , 0.173 moles/L of  $\text{KHCO}_3$ , 0.090 moles/L  $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$  in water.
120. (New): A sperm cell suspension comprising viable spermatozoa and a composition which down-regulates carbohydrate uptake by the spermatozoa, the concentration of spermatozoa in the suspension being less than about  $1 \times 10^6$  or at least about  $1 \times 10^8$  spermatozoa per ml.
121. (New): A sperm cell suspension comprising viable sperm, potassium and optionally sodium, the concentration of spermatozoa in the suspension being less than about  $1 \times 10^6$  or at least about  $1 \times 10^8$  spermatozoa per ml and the molar ratio of potassium to sodium being greater than 1:1, respectively.